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AND TOXIC SUBSTANCES

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

MEMORANDUM

SUBJECT: Carcinogenicity Peer Review of Pronamide (3rd)

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and
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TO: Robert Taylor
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and
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Product Manager #73
Special Review and Reregistration Division (H7508W)

The Health Effects Division Carcinogenicity Peer Review Committee met on September 30, 1992 to discuss and evaluate the weight-of-the-evidence on Pronamide with particular reference to its carcinogenic potential. The Peer Review Committee agreed that Pronamide should be classified as Group B2 - probable human carcinogen with inadequate evidence in humans.

This decision was based on the finding of two types of tumors in the rat (benign testicular interstitial cell tumors and uncommon thyroid follicular cell adenomas), and one type in the mouse (liver carcinomas). The q_1^* will be based on the incidence of liver tumors. The genotoxicity test results were negative, and there were no carcinogenic compounds which were structurally related to Pronamide.

A. Individuals in Attendance:

1. Peer Review Committee: (Signatures indicate concurrence with the peer review unless otherwise stated.)

Karl Baetcke

William L. Burnam

Marcia Van Gemert

Reto Engler

Marion Copley

Kerry Dearfield

Esther Rinde

Jean Parker

Hugh Pettigrew

2. Reviewers: (Non-committee members responsible for data presentation; signatures indicate technical accuracy of panel report.)

Nguyen Thoa[1] 04/06/93

Roger Gardner

Lori Brunsman

(for Lucas Brennecke[2] (PAI/Clement)

3. Peer Review Members in Absentia: (Committee members who were unable to attend the discussion; signatures indicate concurrence with the overall conclusions of the Committee.)

Penelope Fenner-Crisp

Julie Du

George Ghali

Richard Hill

John Quest

William Sette

[1]Also a member of the PRC for this chemical; signature indicates concurrence with the peer review unless otherwise stated.

[2]Signature indicates concurrence with the pathology report.

Yin-Tak Woo Yin Tak Woo

4. Other Attendees:

Eve Anderson (Clement)

B. Material Reviewed:

The material available for review consisted of DERs, one-liners, other data summaries prepared by Nguyen Thoa, and neoplastic tables and statistical analysis by Lori Brunsman. The material reviewed is attached to the file copy of this report. The data reviewed are based on studies submitted to the Agency by Rohm and Haas Co.

C. Background Information:

Pronamide [(3,5-dichloro-N-dimethyl-2-propynyl) benzamide], trade name Kerb®, is a pre- or early post-emergence herbicide which inhibits root and shoot growth from seedlings and is used to control a wide range of annual and perennial grasses as well as certain broadleaf weeds. It is produced and formulated for use as a 50-W wettable powder in water soluble pouches (EPA Reg. No. 707-159) by Rohm and Haas Co., Springhouse, Pennsylvania. It is registered for terrestrial non-domestic food use on alfalfa, apples, globe artichokes, birdsfoot trefoil, blackberries, blueberries, cherries, clover, crown vetch, endive, grapes, lettuce, nectarines, peaches, pears, plums, prunes, raspberries, and sainfoin, for terrestrial non-domestic non-food use on bermudagrass, bermudagrass seed crop, azalea, nursery stock azalea, Christmas tree plantations, Douglas fir, nursery stock Douglas fir, nursery stock fir, forsythia, nursery stock forsythia, holly, nursery stock holly, juniper, nursery stock juniper, pine, nursery stock pine, rhododendron, nursery stock rhododendron, yew, and nursery stock yew, and for domestic outdoor uses on bermudagrass, centipedegrass, St. Augustinegrass, and zoysiagrass.

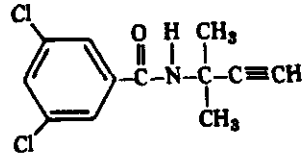
Following the Data-Call-In Notice of the first Registration Standard of 1986, new toxicity studies were received including a chronic feeding/oncogenicity study in rats, a 15-week thyroid hormonal study in rats, a 13-week testicular hormonal pilot study in rats, a developmental study in rats, seven mutagenicity studies covering gene mutation, structural chromosomal aberration, and other genotoxic effects, a general metabolism study in rats, and a dermal penetration study in rats. An additional testicular hormonal study will be available for review in 1993.

Pronamide is an off-white powder, practically insoluble in water (15 ppm at 25° C), freely soluble in acetone, with a melting point of 155-156° C, and a vapor pressure of 8.5×10^{-5} mm Hg at 25° C.

The Caswell (or Tox Chem) Number of Pronamide is 306A.

The Chemical Abstracts Registry Number (CAS No.) is 23950-58-5.

The structure of Pronamide is



A Rebuttable Presumption Against Registration (RPAR) Notice for products containing Pronamide was issued on May 20, 1977 by the Agency, based on observation of liver carcinomas in an acceptable chronic feeding/oncogenicity study conducted with B6C3F1 mice [Medical College of Virginia (MCV) 1974 study]. Cancer risks (q_1^*) estimated by the Agency's Carcinogenic Assessment Group (CAG), with the use of a one-hit model to estimate "slopes" for a linearized low-dose extrapolation, were 1.71×10^{-4} $(ppm)^{-1}$ and 1.81×10^{-4} $(ppm)^{-1}$ for the doses of 1000 and 2000 ppm, respectively. Results of the 1974 study were confirmed by a different testing laboratory (MIT 1982 study).

The first carcinogenicity Peer Review meeting on Pronamide took place on February 4, 1985. Upon evaluation of the carcinogenicity data from both the MCV and MIT studies, the committee concluded that "the overall assessment of Pronamide was not substantially affected when considering the results of the MIT mouse study, i.e., the previous conclusions of the Agency's RPAR process remain supported, unchanged and valid." Pronamide was consequently classified as a Group C oncogen (possible human oncogen). Cancer risks were estimated based on the acceptable 1974 MCV study, but with the use of the Crump's multi-hit Global 83 program. The estimated q_1^* was 1.63×10^{-2} $(mg/kg/day)^{-1}$ [q_1^* based on the MIT study was estimated to be 5.19×10^{-2} $(mg/kg/day)^{-1}$].

The carcinogenicity data base supporting the Agency's decision to classify Pronamide as a Group C oncogen with a q_1^* was reviewed by a FIFRA SAP on May 22, 1986. The panel agreed with EPA's classification of Pronamide as a category "C" oncogen but disagreed with a quantitative risk assessment because "it is not justified to make quantitative risk assessment for any

pesticides classified in Groups C or D in regards to oncogenicity, unless there are mitigating circumstances."

A second carcinogenicity Peer Review committee on Pronamide met on March 22, 1988 to consider SAP's advice. The Committee disagreed with SAP's conclusions and maintained that "the data on Pronamide supported a risk quantitation because (1) there was a dose response with respect to tumor induction, and (2) there were clearly malignant liver tumors observed." The committee requested a rat chronic feeding/oncogenicity study; pending an evaluation of the carcinogenicity data from the requested study, the PRC maintained a tentative classification for Pronamide as a category "C" oncogen, with a q_1^* of 1.63×10^{-2} $(mg/kg/day)^{-1}$. The requested rat study has been reviewed and the carcinogenic data are now presented for evaluation.

D. Evaluation of Carcinogenicity Data

1. Sprague-Dawley Rat Chronic Feeding/Oncogenicity Study

Reference: Bailey, R.H. 1990. Kerb® Herbicide (Technical, no clay): 24-Month Dietary Chronic Toxicity/Oncogenicity Study in Rats. MRID Nos. 417140-01 (12-month phase) and 417140-02 (24-month phase). Hazleton Laboratories America, Inc., Vienna, VA. Studies No. HLA 417-426S and 417-426M.

a. Experimental Design

Kerb® technical [96.4% active ingredient (a.i.)] was administered in the diet to groups of 60 male and 60 female Crl:CD (SD)BR rats at 0 (control), 40, 200, or 1000 ppm (0, 1.73, 8.46, or 42.59 mg/kg/day/male and 0, 2.13, 10.69, or 55.09 mg/kg/day/female) for 24 months (24-month phase). Ten extra animals/sex/dose group were assigned to be sacrificed after 6 and 12 months of treatment (12-month phase).

b. Discussion of Tumor Data

Statistical analysis of tumor rates was based on the Cochran-Armitage Trend Test and Fisher's Exact Test for pair-wise comparison of controls and each treated group since there was no significant statistical evidence of differential mortality with increasing doses of Pronamide.

At 1000 ppm, in the 24-month phase, both male and female rats had increased rates of thyroid follicular cell adenomas (Table 1), and male rats had an increased incidence of benign testicular interstitial cell tumors (Table 2). Thyroid tumors were not observed until weeks 53 and 82 for males and females,

respectively, and testicular tumors were not observed until week 67. The increase in thyroid tumor rate was statistically significant by pair-wise comparison ($p < 0.01$) only in males, but there was a positive trend ($p < 0.01$) for both sexes. Both high dose male and female tumor rates (21% and 10%, respectively) exceeded the historical control range which was 0-14.8% (mean 5%) for males and 0-9.5% (mean 2%) for females (Hazleton Laboratories, Vienna, VA: historical control data for SD rats obtained from 13 studies conducted between 1985 and 1990). There were no significant differences in thyroid follicular cell carcinoma rates between groups. There were increasing trends and/or rates in combined thyroid follicular cell adenomas and carcinomas (trend $p < 0.01$ in males, $p < 0.05$ in females; pair-wise comparison of high dose males/controls, $p < 0.05$) which were a reflection of the treatment-related changes in thyroid follicular cell adenoma rates. The increase in testicular interstitial cell benign tumor rate was statistically significant by pair-wise comparison ($p < 0.05$) and there was a positive trend ($p < 0.01$). In high dose males, the tumor rate (27%) exceeded the historical control range of 4.8-18.2% with a mean value of 5.6% (Hazleton Laboratories, Vienna, VA: historical control data for SD rats obtained from 11 studies conducted between 1985 and 1990). In the 12-month phase, thyroid follicular cell and testicular interstitial cell neoplasia were not observed in any group.

Benign pituitary adenomas of the pars distalis were observed in every dose group during both the 12- and 24-month phases, but the tumor rates were statistically comparable among all groups. The respective tumor rates for the 0, 40, 200, and 1000 ppm dose groups were 1/19, 0/19, 0/20, and 3/20 in males and 0/20, 2/20, 1/19, and 3/20 in females in the 12-month phase and 31/60, 33/60, 35/60, and 34/60 in males and 49/60, 49/60, 49/60, and 54/60 in females in the 24-month phase.

Table 1. Thyroid Follicular Cell Tumor Rates[+] and Cochran-Armitage Trend Test and Fisher's Exact Test Results (p values).

	Dose (ppm)			
	0	40	200	1000
Tumors		Males		
Adenomas	4/68	2/70	6/69	14[a]/67
(%)	(6)	(3)	(9)	(21)
p =	0.000**	-	0.382	0.009**
Carcinomas	4/68	2[b]/70	1/69	4/67
(%)	(6)	(3)	(1)	(6)
p =	0.243	-	-	0.633
Combined	8/68	4/70	7/69	18/67
(%)	(12)	(6)	(10)	(27)
p =	0.000**	-	-	0.022*
		Females		
Adenomas	1/59	2/58	1/58	6[c]/59
(%)	(2)	(3)	(2)	(10)
p =	0.006**	0.494	0.748	0.057
Carcinomas	1[d]/59	1/58	0/58	0/59
(%)	(2)	(2)	(0)	(0)
p =	0.155	0.748	-	-
Combined	2/59	3/68	1/58	6/59
(%)	(3)	(5)	(2)	(10)
p =	0.028*	0.492	-	0.136

[+] Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 53 (males) or week 54 (females).

[a]First adenoma in males observed at week 53, dose 1000 ppm.

[b]First carcinoma in males observed at week 83, dose 40 ppm.

[c]First adenoma in females at week 82, dose 1000 ppm.

[d]First carcinoma in females at week 105, dose 0 ppm.

Note: Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 2. Male Testes Interstitial Cell Benign Tumor Rates[+] and Cochran-Armitage Trend Test and Fisher's Exact Test Results (p values).

	Dose (ppm)			
	0	40	200	1000
Rate	5/58	5/60	3/59	15[a]/56
(%)	(9)	(8)	(5)	(27)
p =	0.000**	0.607	-	0.010*

[+] Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 54.

[a]First testes interstitial cell benign tumor observed at week 67, dose 1000 ppm.

Note: Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$

c. Non-Neoplastic Lesions (Table 3)

Increased incidences of non-neoplastic lesions were observed in the liver, thyroid, and ovaries of high-dose rats.

In the liver, a positive trend ($p < 0.01$) in the incidence of centrilobular hypertrophy was observed in males and females in both phases (12- and 24-month); the increases observed at 1000 ppm were significant by pair-wise comparison ($p < 0.01$, both sexes) and appeared more pronounced in the 12-month phase (rate = 65% in males and 95% in females) than in the 24-month phase (rate = 20% in males and 48% in females). Hypertrophy was accompanied by eosinophilic cell alteration in the 24-month phase [$p < 0.01$ for positive trend in both sexes; pair-wise comparison in high dose/controls, $p < 0.05$ (males) and $p < 0.01$ (females)].

In the thyroid, a positive trend ($p < 0.05$ in males and $p < 0.01$ in females) in the incidence of follicular hypertrophy was observed in the 12-month phase but not the 24-month phase. The increased incidence observed at 1000 ppm was only significant ($p < 0.01$, high dose/controls) in females. In the 24-month phase, a positive trend ($p < 0.01$) in the incidence of follicular hyperplasia was observed in females, but the increased incidence observed at 1000 ppm was not statistically significant.

In the ovaries, a positive trend ($p < 0.01$) in the incidence of sertoliform tubular hyperplasia was observed in females in the 24-month phase, and the increase in incidence observed at 1000 ppm was significant ($p < 0.01$) by pair-wise comparison.

Table 3. Non-Neoplastic Lesion Rates[+] and Cochran-Armitage Trend Test and Fisher's Exact Test Results (p values)

	Dose (ppm)							
	Males				Females			
	0	40	200	1000	0	40	200	1000
12-Month Phase								
Liver Centrilobular Hypertrophy	0/20	0/20	0/20	13/20	0/20	0/20	0/20	19/20
p =	**			**	**			**
Thyroid Follicular Hypertrophy	13/20	5/20	8/20	15/20	2/20	2/20	2/20	12/20
p =	*	*			**			**
24-Month Phase								
Liver Centrilobular Hypertrophy	0/60	0/60	0/60	12/60	1/60	0/20	0/59	29/60
p =	**			**	**			**
Eosinophilic cell Alteration	13/60	8/60	10/60	24/60	5/60	2/60	5/59	18/60
p =	**			*	**			**
Thyroid Follicular Hyperplasia	1/60	0/60	0/60	1/60	0/60	2/60	0/60	4/59
p =					*			
Ovary Sertoliform Tubular Hyperplasia					21/60	19/59	22/60	38/60
p =					**			**
Testes Interstitial Cell Hyperplasia	2/60	7/60	3/60	5/59				
p =								

[+] Number of lesion bearing animals/Number of animals examined (animals sacrificed at term plus those that were sacrificed moribund).

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

d. Adequacy of Dosing for Assessment of Carcinogenic Potential

The dosing was considered to be adequate for assessing the carcinogenic potential of Pronamide, based on body weight gain depressions ($p < 0.05$) of ≥10% observed at 1000 ppm (weeks 0-26 in males; weeks 0-52 in females). Feed consumption was also depressed ($p < 0.05$) at 1000 ppm in males during weeks 1-13 (7% below control) and in females during weeks 1-13 (7%), 1-26 (7%), and 1-52 (5%).

Survival rate was comparable between groups. The statistical evaluation of mortality indicates no significant incremental changes with increasing doses of Pronamide in either male or female rats.

2. 18-Month Carcinogenicity Study in B6C3F1 Mice (MCV 1974 Study)

Reference: Smith, J. 1974. Eighteen-Month Study of the Carcinogenic Potential of Kerb® (RH-315; Pronamide) in Mice. Study conducted at the Medical College of Virginia (MCV) for Rohm and Haas Company. MRID No. 107968.

This study was previously reviewed (1977 CAG, 1985 and 1988 Peer Reviews) and considered to be adequate for a determination of carcinogenic potential.

a. Experimental Design

Kerb® technical (97% a.i.) was administered in the diet to groups of 100 male and 100 female B6C3F1 mice at 0 (control), 1000, or 2000 ppm for 18 months. Additional groups of 25 mice/sex/dose were assigned to the 6-month interim sacrifice.

b. Discussion of Tumor Data (Table 4)

A dose-related increase in the incidence of hepatocellular carcinomas was observed in male mice sacrificed at termination (18 months). The increases in tumor rates observed at 1000 and 2000 ppm were both statistically significant by pair-wise comparison with controls ($p < 0.01$). Pronamide did not induce hepatocellular carcinomas in female mice (rates at termination were 0/100 in controls; 1/100 at 1000 ppm; 2/100 at 2000 ppm). Survival rates of all groups were comparable (≥90%).

Table 4. Male Hepatocellular Carcinomas Rates[+] and Cochran-Armitage Trend Test and Fisher's Exact Test Results (p values).

	Dose (ppm)		
	0	1000	2000
Tumor	7/100	18/100	24/99
p =	**	*	**

[+] Number of animals with tumors/Number of animals examined.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

c. Adequacy of Dosing for Assessment of Carcinogenic Potential

The dosing was considered to be adequate for assessing the carcinogenic potential of Pronamide, based on body weight gain depressions in high dose females (16.5% decrease, weeks 2-78, $p < 0.05$), and increases in relative body weight of the liver at ≥1000 ppm in both sexes [23% at 1000 ppm and 41% at 2000 ppm, $p < 0.05$ (males); 14% at 1000 ppm and 36% at 2000 ppm, $p < 0.05$ (females)].

3. 24-Month Carcinogenicity Study in B6C3F1 Mice (MIT 1982 Study)

Reference: Newberne, P. M. et al. 1982. Chronic Toxicity Study in the Mouse. Final Report No. 81RC-157 conducted at the MIT Animal Pathology Labs. for Rohm and Haas. EPA Acc. No. 248233.

This special study was conducted with male mice. Its purpose was to determine the toxicological significance of liver lesions observed in male mice in the MCV 1974 study. This study was graded core supplementary since it only used male animals, and did not meet GLP standards for record-keeping. This study was reviewed at the first Peer Review on Pronamide. The results are briefly described below.

a. Experimental Design

Kerb® technical (>93.8% a.i.) was administered in the diet to groups of 63 male B6C3F1 mice at 0 (control 1), 0 (control 2), 20, 100, 500, or 2500 ppm for 24 months. Additional groups of mice were assigned to interim sacrifices at 6 months (42 at 0 ppm; 42 at 2,500 ppm) and at 15 and 18 months (42/group including controls 1 and 2, and 20, 100, 500, and 2500 ppm groups).

b. Discussion of Tumor Data (Table 5)

This study confirmed the results of the MCV 1974 study; long term (24 months) exposure of male mice to Pronamide was associated with an increased incidence of hepatocellular carcinomas (Table 5). A positive trend ($p < 0.05$) in incidences of hepatocellular carcinomas was observed in mice sacrificed at 24 months, and the increased tumor rates observed at ≥100 ppm were statistically significant ($p < 0.05$ at 100 ppm; $p < 0.01$ at 500 and 2500 ppm). Hepatocellular adenomas were not observed in the MCV study but there was both a positive trend ($p < 0.01$) and pair-wise differences at 2500 ppm ($p < 0.01$) in mice sacrificed at 24 months in this study. There also appeared to be a progression from benign to malignant tumors. Survival rates were excellent for all groups (≥93%).

Table 5. Male Hepatocellular Tumor Rates[+] and Cochran-Armitage Trend Test and Fisher's Exact Test Results (p values).

			Dose (ppm)				
Tumor:	0_1 Control 1	0_2 Control 2	$0_1 + 0_2$	20	100	500	2500
6-month	0/42	-	-	-	-	-	0/42
15-month							
Adenomas	2/42	2/42	4/84	1/42	2/42	4/42	3/42
Carcinomas	3/42	0/42	3/84	1/42	2/42	2/42	1/42
18-month							
Adenomas	3/42	3/42	6/84	4/42	4/42	2/41	6/41
Carcinomas	4/42	2/42	6/84	3/42	3/42	4/41	6/42
24-month							
Adenomas	4/63	6/63	10/126	6/63	7/63	8/63	28/61
p =			**				**
Carcinomas	5/63	5/63	10/126	9/63	12/63	18/63	14/61
p =			*		*	**	**

[+] Number of tumor bearing animals/Number of animals examined.
- Not done.

Note: Significance of trend denoted at combined controls 1+2.
Significance of pair-wise comparison denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

c. Non-Neoplastic liver Lesions (Table 6)

Male mice treated with the high dose (2500 ppm) for 24 months showed increased incidences of gross liver lesions (liver enlarged, with nodules/mass) and microscopic liver lesions (hypertrophy, parenchymal necrosis, and cholestasis). Statistical analysis of these data is not available.

Table 6. Incidences of Gross and Non-Neoplastic Liver Lesions in Male Mice

	Dose (ppm)					
	O_1 Control 1	O_2 Control 2	20	100	500	2500
Gross Lesions						
Enlarged	5/63	1/63	3/63	2/63	17/63	55/63
Nodules/Mass	34/63	32/63	29/63	39/63	50/63	58/63
Non-Neoplastic Microscopic Lesions						
Hypertrophy	26/63	26/63	45/63	23/63	24/63	51/61
Hyperplastic Nodules	23/63	23/63	14/63	23/63	25/63	19/61
Cholestasis	0/63	0/63	0/63	0/63	0/63	55/61
Parenchymal Necrosis	3/63	4/63	4/63	1/63	1/63	21/61

d. Adequacy of Dosing for Assessment of Carcinogenic Potential

The dosing was considered to be adequate for assessing the carcinogenic potential of Pronamide, based on decreased body weight (30%, months 6-24) and increased liver weight (30-40% absolute (abs) weight increase; 100% rel body weight increase) in the high dose group.

E. Additional Toxicological Data

1. Hormonal Studies

a. Addendum to Rat Chronic Feeding/Carcinogenicity Study (Bailey et al. 1990)

Reference: Hazelton, G.A., et al. 1991. Pronamide (Kerb® Herbicide): Thyroid Function and Hepatic Clearance in Male Rats. MRID No. 420934-01. Rohm and Haas Tox. Dept. Springhouse, PA. Studies No. 90R-178. Submitted in support for a Pronamide's thyroid tumorigenic effect involving a disruption of the pituitary-thyroid hormonal balance.

i. Experimental Design

Kerb® technical (96.4% a.i.) was administered in the diet to groups of male Crl:CD (SD)BR rats at 0 (control), 40, 1000, or 4000 ppm (about 0, 3, 67, or 279 mg/kg/day) according to the following experimental schedule:

Test group	ppm a.i. in diet	Total No. rats/group	No. dosed for 4 wks	No. dosed for 15 wks	Recovery Period (wks)
1 (Control)	0	40	20	20	0
2 (LDT)	40	20	10	10	0
3 (MDT)	1000	20	10	10	0
4 (HDT)	4000	40	20	20	0
5 (Recovery)*	4000	20	20	-	11

* 4000 ppm for 4 wks then 0 ppm for the following 11 weeks.

Serum L-thyroxine (T4), L-triiodothyronine (T3), reverse triiodothyronine (rT3), thyroid stimulating hormone (TSH), SGOT, and SGPT, and pathology of the thyroid and pituitary were determined in 10 rats/groups 1-4 after 4 or 15 weeks of treatment and 10 rats/group 5 at week 15. Liver of these rats (8 rats/groups 1, 4, and 5) were also assayed for microsomal UDP-glucuronosyl transferase (UDP-GT) activity. Bile flow and biliary excretion of ^{125}I-T4 and ^{125}I-T4 glucuronide were determined in 10/groups 1 and 4 after 4 or 15 weeks of treatment and 10 rats/group 5 at 15 week.

ii. Results

a) Histopathology of the Thyroid (Table 7)

Treatment with 1000 and 4000 ppm Pronamide for 4 or 15 weeks was associated with similar increases ($p < 0.05$) in incidence of diffuse hypertrophy/hyperplasia of the thyroid follicular cells. There was a positive trend after both 4 and 15 weeks. The lesions were observed throughout the thyroid, and were characterized by follicular cells with increased height, and by follicles reduced in size and in colloid content. The increase in incidences observed after 4 weeks of treatment with the high dose (10/10) was reduced (5/9) after 11 weeks of recovery but did not disappear in all animals after the recovery period. The severity of the lesions was greater at 4000 ppm than at 1000 ppm.

Table 7. Incidences of Thyroid Follicular Cell Hypertrophy/Hyperplasia in Male Rats.

	Dose (ppm)			
Treatment (weeks)	0	40	1000	4000
4	2/10*	4/10	10/10*	10/10*
15	3/10*	5/10	9/10*	10/10*
4 + 11 Wks Recovery	-	-	-	5/9

Note: Significance of trend denoted at control.
Significance of pair-wise comparison denoted at dose level.
If *, then $p < 0.05$.

b) Serum levels of TSH, T4, T3, and rT3 (Table 8)

Treatment with 40-4000 ppm Pronamide for 4 or 15 weeks was not associated with any reduction in T3 or rT3. T4 was moderately reduced (decrease at 1000 ppm: 61% after 4 weeks and 48% after 15 weeks; decrease at 4000 ppm: 87% after 4 weeks and 84% after 15 weeks). Except for a moderate increase (72%) observed after 4 weeks of treatment with 1000 ppm, TSH remained unaffected. The decrease in T4, which was observed after 4 weeks of treatment with 4000 ppm, was absent after 11 weeks of recovery. Serum SGOT and SGPT were not affected by Pronamide.

Table 8. Serum TSH, T/4, T3, and rT3 in Male Rats

Group	ppm		TSH μunit/ml	T4 μg/dl	T3 ng/dl	rT3 pg/ml
4 week dosing						
1	0	Mean	110.72	6.99	77.43	101.67
		SEM	14.49	0.57	3.57	22.73[a]
2	40	Mean	115.31	7.23	97.52*	132.05
		SEM	14.79	0.63	4.18	20.39
3	1000	Mean	190.49*	2.74*	77.56	107.93
		SEM	31.41	0.12	4.87	14.74
4	4000	Mean	154.65	0.94*	75.80	169.09
		SEM	15.87	0.07	4.29	27.79[a]
15 week dosing						
1	0	Mean	84.66	6.99	83.06	87.83
		SEM	5.79	0.46	5.93	15.17[a]
2	40	Mean	108.77	5.44	81.95	113.39
		SEM	14.54	0.30	7.82	12.72
3	1000	Mean	123.58	3.58*	75.27	125.05
		SEM	20.98	0.26	4.12	24.24
4	4000	Mean	123.59	1.15*	83.11	144.63
		SEM	14.14	0.12	3.42	17.86
4 week dosing + 11 week recovery						
5	4000	Mean	94.67	6.46+	92.77	120.50
		SEM	8.69	0.32	3.66	20.48

10 animals/group except [a] = 9 animals/group.

* Treated vs Control, $p < 0.02$.

+ Recovery vs HDT (ANOVA + Dunnett's T-test), $p < 0.02$.

c) Hepatic UDP-GT Activity

Treatment with 4000 ppm Pronamide for 4 or 15 weeks was associated with a 2-2.5 fold increase in UDP-GT activity (activity was expressed as nmol T4-glucuronide formed/min/μg liver microsomal protein). The increase observed after 4 weeks of treatment with 4000 ppm was absent after 11 weeks of recovery.

d) Bile Flow and Biliary clearance of ^{125}I-T4 (Table 9)

Treatment with 4000 Pronamide ppm for 4 or 15 weeks was associated with significant ($p < 0.05$) increases in bile flow

(≤65%), biliary clearance of ^{125}I-T4 (7-10 fold) and ^{125}I-T4 glucuronide (1-2 fold), and ^{125}I bile/plasma ratio (4-5 fold). The increases in bile flow, biliary clearance of ^{125}I-T4 and ^{125}I-T4 glucuronide, and ^{125}I bile/plasma ratio, observed after 4 weeks of treatment, were completely reversed after 11 weeks of recovery.

Table 9. Parameters of Biliary Excretion in Male Rats Treated with 4000 ppm Pronamide; percent increase over control.

	Bile flow[a]	^{125}I-T4 Clearance[a]	^{125}I Ratio Bile/Plasma[a]	^{125}I-T4 Glucuronide Clearance[b]
Treatment (Weeks)		%		
4	63*	999*	540*	203*
15	55*	778*	455*	158*
4 + 11 Wks Recovery	15+	4+	0+	0+

[a] % increases at [(0-30)+(30-60)+(60-90)+(90-120)+(120-180)+(180-240)]/6 minutes.
[b] Cumulative % increases over the entire 4-hour collection.

* $p < 0.05$, 4000 ppm group vs. Control group (Student's t-test).
+ $p < 0.05$, Recovery group vs. 4000 ppm group (Student's t-test).

iii. Adequacy of Dosing for Assessment of Thyroid Effects

The dosing was considered to be adequate for assessing the thyroid effects of Pronamide, based on significant ($p \leq 0.05$) depressions of body weight (2-5% at 1000 ppm, weeks 1-4; 17-24% at 4000 ppm, weeks 1-15) and feed consumption (4-10% at 1000 ppm, weeks 1-4; 11-38% at 4000 ppm, weeks 1-15). Absolute (abs) and/or relative (rel) liver weight was significantly increased ($p \leq 0.05$) at ≥1000 ppm, in a dose-related manner (4 week increases: 29% abs and 36% rel at 1000 ppm, 50% abs and 91% rel at 4000 ppm; 15 week increases: 32% rel at 1000 ppm, 42% abs and 86% rel at 4000 ppm). Thyroid weight was significantly increased ($p \leq 0.05$) at ≥1000 ppm in a non dose-related manner [4-week increases: 29% abs and 36% rel at 1000 ppm; 32% rel at 4000 ppm. 15 week increases: 21% rel at 1000 ppm; 33% rel at 4000 ppm).

b. Addendum to Rat Chronic Feeding/Carcinogenicity Study (Bailey et al. 1990)

Reference: Hazelton, G.A., et al. 1991. Pronamide ("Kerb® Herbicide): Effects on Endocrine Regulation of the Testis in Rats - Pilot Study. MRID No. 421396-01. Rohm and Haas Tox. Dept. Springhouse, PA. Studies No. 90R-179. Submitted in support

for a Pronamide's testicular tumorigenic effect involving a disruption of the pituitary-thyroid hormonal balance.

i. Experimental Design

Kerb® technical (96.4% a.i.) was administered in the diet to groups of 20 male Crl:CD (SD)BR rats at 0 (control) or 4000 ppm (0 or 273 mg/kg/day) for 13 weeks. At termination, the serum was assayed for LH, FSH, prolactin, testosterone, dihydrotestosterone, estradiol, estrone, and corticosterone, and the liver was assayed for several microsomal enzymes. The pituitary, testes, sex organs, and epididymides were examined for pathology. Additional groups of 10 rats were fed diets containing 0 (control), 40, 1000, or 4000 ppm for 4 weeks to determine serum LH, FSH, testosterone, and prolactin.

ii. Results

a) Histopathology of the Testes

Treatment with 4000 ppm Pronamide for 13 weeks was associated with an increase in the number of testicular interstitial cells. The incidences were 1/20 for the control group and 7/20 for the treated group. Interstitial cells are located between the seminiferous tubules, normally in 2-3 cell thick layers. An increase in the number of testicular interstitial cells is stated to have occurred when they form focal clusters instead of layers. In this study, the observed increase was determined to be equivocal because of "the small degree of change being evaluated and the possibility of producing this appearance through fortuitous tangential sectioning of seminiferous tubules." The PRC determined that this type of sectioning could be seen in all dose groups.

b) Clinical Chemistry Changes (Table 10):

Treatment with 4000 Pronamide ppm for 4 or 13 weeks was associated with increases in serum LH and FSH. The increases were moderate and were slightly higher at 4 weeks than at 13 weeks (respective increases at 4 and 13 weeks were 60% and 58% for FSH, and 100% and 77% for LH). Serum LH and FSH levels were not affected by the mid-level dose (1000 ppm). Serum testosterone was not affected by Pronamide.

Table 10. Increase (% over control) in Serum LH, FSH, and Testosterone Levels in Male Rats.

	LH	FSH	Testosterone
13-Week Dosing[a]			
ppm a.i.			
4000	77%*	58%*	NS
4-Week Dosing[b]			
40	NS	NS	NS
1000	NS	NS	NS
4000	100%*	60%*	NS

Hormone levels expressed as ng product/ml.

[a] Samples from 18-20 rats/group.

[b] Samples from 10 rats/group.

* $p \leq 0.05$ Treated vs. Control (Student's t-test).

NS: Not statistically significant ($p < 0.05$, Student's t-test).

c) Liver Microsomal Enzymes Activity (Table 11)

Treatment with 4000 ppm Pronamide for 13 weeks increased the activity of the liver microsomal enzymes cytochrome-P_{450}, cytochrome-B_5, and NADPH-cytochrome C reductase and the rate of oxidation of testosterone, expressed as μmol product/whole liver. Concomitant increases in liver weight (50%) and liver microsomal protein content (34%) were observed, which suggests that oral administration of relatively high doses of Pronamide (4000 ppm) for a certain period of time (13 weeks) may result in induction of the liver enzymes responsible for its metabolism.

Table 11. Increase (% over control) in Liver Microsomal Enzymes Activity and Oxidation Rate of Testosterone after 13 Weeks of Dosing with Pronamide 4000 ppm.

	Oxidized Testosterone	Cytochrome-P_{450}	Cytochrome-B_5	NADPH-Cytochrome C Reductase
nmol Product /mg Protein	13%*	0%	70%*	157%*
nmol Product /g Liver	54%*	-	-	-
μmol Product /Whole Liver	137%*	100%*	263%*	444%*

Data based on 8 control and 8 treated rats.
Pronamide 4000 ppm increased abs liver weight by 56% ($p < 0.05$) and microsomal protein content by 34% ($p < 0.05$).
- Not done.
* $p \leq 0.05$ Treated vs. Control (Student's t-test).

iii. Adequacy of Dosing for Assessment of Testicular Effects

The dosing (4000 ppm) was considered to be adequate for assessing the testicular effects of Pronamide, based on significant ($p \leq 0.05$) depressions of body weight (14-17%, weeks 1-13) and feed consumption (37%, week 1; 9-12%, weeks 2-8). Testicular relative (to body) weight was slightly increased (26%; $p < 0.05$) and liver absolute and relative (to body) weight were moderately increased (59% abs, 92% rel; $p \leq 0.05$). The liver-related enzymes SGPT and SGOT were not affected.

2. Pharmacokinetic Studies

References: Didonato, L. J. and G. A. Hazelton. 1991. ^{14}C-Pronamide (Kerb® Herbicide): Pharmacokinetics Study in Rats. Rohm and Haas Study No. 89R-163. MRID No. 418018-01.

Smith Jr. S. 1991. Rat Metabolism of ^{14}C-Pronamide. Rohm and Hass Study No. 34-91-43. MRID No. 419299-01.

Male and female Crl:CD®BR rats received a single dose of 2 or 100 mg/kg ^{14}C-Pronamide by gavage. The test material was moderately absorbed and completely and rapidly eliminated. Over 93% of the dose at both dose levels was excreted in the urine and feces over 7 days. Peak plasma concentrations occurred within the first 8 hours after dosing (1st sampling time = 8 hours post-dose). The label was detected throughout the body with the highest concentrations (in decreasing order) in fat, adrenal,

bone marrow, thyroid, liver, kidney, and plasma. No bioaccumulation was evident in any tissue.

Following a low dose, urinary excretion of label was comparable to fecal excretion in males (47% dose in urine; 46% dose in feces) but was higher than fecal excretion in females (57% dose in urine; 40% dose in feces). Following a high dose, urinary excretion of label was lower than fecal excretion in both sexes (35-39% dose in urine; 57-60% dose in feces). Results from previous studies showed that no label was exhaled as $^{14}CO_2$ during the first 48 hours after dosing.

No sex difference was apparent in the rate of excretion of the test material. Plasma half life of a low dose was biphasic [rapid (α) phase = 12.6 hours (males) and 12.7 hours (females); slow phase (β) = 36.6 hours (males) and 45.3 hours (females)], and that of the HD rats was monophasic [$t_{1/2}$ = 24.1 hours (males) and 24.8 hours (females)].

The feces were not examined for metabolites. Very little unchanged Pronamide (≤0.4% total urinary radioactivity) was recovered in urine. Twenty urinary radioactive metabolites were found but those that were clearly identified/quantified constituted only 19.3-51.1% of the total urinary radioactivity. These 2 complementary studies did not totally satisfy the guideline requirement for a general metabolism study.

There is no acceptable dermal absorption study with Pronamide.

3. Mutagenicity Studies

Results of mutagenicity studies with an acceptable core classification are briefly summarized in Table 12.

Table 12. Mutagenicity Studies With Pronamide

Study Type	MRID No.	Results
Gene Mutation (Ames)	400906-02	Negative, with or without metabolic activation.
Forward Gene mutation	402111-06	Negative, with or without metabolic activation, in CH V79 cells.
Structural Chromosome Aberration in-vitro	402111-08	Negative with or without metabolic activation, in CHO cells.
Structural Chromosome Aberration in-vivo	402111-05	Negative in mouse bone marrow cells.
Unscheduled DNA Synthesis	402111-07	Negative in primary rat hepatocytes

4. Structure-Activity Correlations

A computerized structure-activity search (Chemline), performed for Pronamide, was negative for carcinogenic results and/or mutagenic activity. According to Woo and his associates (Woo, Y. T. et al.: Novel Types of Carcinogens. In: Chemical Induction of Cancer - Structural Bases and Biological Mechanisms, Vol. IIIB, Aliphatic and polyhalogenated Carcinogens, Appendix I, pp 463-480, Academic Press Inc., New York, 1985), Pronamide "represents a novel structural type compared with the classes of known carcinogens."

5. Acute, Subchronic, and Chronic Toxicity Studies

There is no acceptable available subchronic oral toxicity study in rats. In a study core classified "Supplementary", groups of CD rats (10/sex/dose group) received 0, 50, 150, 450, 1350, or 4050 ppm Pronamide in their diet for 3 months. There were no adverse effects on hematology or urinalysis parameters and no histological lesions. A NOEL was established at 50 ppm and a LOEL at 150 ppm, based on significant ($p < 0.05$) increases in liver absolute and relative weight (17% abs, 11% rel in males; 29% abs, 18% rel in females). The high dose caused decreases in body weight (22%, week 1 and 13%, week 2, in males; 11-20%, weeks 1-13, in females) and increases in liver weight in both sexes(11% rel in males; 29% abs and 50% rel in females), and increases in testes weight in males (13% abs, 18% rel). The core classification of this study was based on an absence of any individual data (body weight, feed consumption, hematology, urinalysis, and organ weight), clinical observations,

ophthalmologic examinations, or analysis for test material concentration/stability in the diet.

F. Weight-of-Evidence Considerations

The PRC considered the following facts regarding the toxicology data on Pronamide in a weight-of-the-evidence determination of carcinogenicity potential for Pronamide:

1. Pronamide exposure was associated with a dose-related increase in the incidence of liver carcinomas in male B6C3F1 mice in a chronic feeding/carcinogenicity study (MCV 1974 Study). This finding was later confirmed in a special study conducted at a different laboratory (MIT 1982 study). In both studies, the tumors occurred late in the lifespan of test mice. There was an apparent progression from adenomas to carcinomas.

2. Pronamide exposure was associated with statistically significant increases in the incidence of thyroid follicular cell adenomas in male rats (pair wise comparison at the high dose). There was a positive trend in the incidence of thyroid follicular cell adenomas in male and female rats. The incidence of thyroid follicular cell adenomas exceeded historical control ranges.

3. The PRC considered the mechanistic studies the Registrant submitted concerning hormonal disturbance.[3] They concluded that the evidence did not definitively attribute a hormonal imbalance mechanism for the thyroid tumors associated with administration of Pronamide.

4. The incidence of benign testicular interstitial cell tumors in high dose male Crl:CD (SD)BR rats was increased by trend and pair-wise analysis. The incidence at the high dose exceeded historical control ranges. Most of the tumors (14/15) occurred late in the life-span of the animal and were located at terminal sacrifice.

It is clear from the non-neoplastic lesions and chronic studies that liver and thyroid are primary targets of Pronamide toxicity consistent with findings of tumors at these sites.

5. Tests for genotoxicity were negative.

6. A computerized structure-activity search (Chemline) showed no chemicals with carcinogenic and/or mutagenic activity which were structural analogs of Pronamide.

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[3]For a more detailed discussion on these special studies, see R. Gardner Memo 3/18/93, attached to the file copy of this report.

G. Classification of Carcinogenic Potential:

The Peer Review Committee considered the criteria contained in the EPA's "Guidelines for Carcinogen Risk Assessment" [FR 51: 33992-34003, 1986] for classifying the weight of evidence for carcinogenicity.

The Peer Review Committee agreed that the classification for Pronamide should be Group B2 - probable human carcinogen with inadequate evidence in humans. This decision was based on the finding of two types of tumors in the rat (benign testicular interstitial cell tumors and uncommon thyroid follicular cell adenomas), and one type in the mouse (liver carcinomas). The q_1^* will be based on the incidence of liver tumors. The genotoxicity test results were negative, and there were no carcinogenic compounds which were structurally related to Pronamide.

The PRC considered the work of the registrant in attempting to show a hormonal mechanism for the thyroid tumors, but determined that even if the rat thyroid tumors are ascribed to hormonal mechanisms, the mouse liver tumors can not be discounted. The submitted data on thyroid tumor formation are suggestive of a hormonal mechanism but are not conclusive. The classification of Pronamide, though consistent with current policy, may change at a later date pending development of Agency guidance on the tumor combination (e.g. mouse liver tumors with rat thyroid tumors) and on rat testicular tumors. New hormonal data associated with induction of testicular tumors are forthcoming, which may also change the present classification of Pronamide.

H. Support for Hormonal Mechanism

1. Threshold Model for Thyroid Neoplasm

a. Consideration of the use of the Threshold Model for Pronamide

The committee considered the possibility of using the threshold model for thyroid neoplasm for Pronamide. The discussion which follows was taken from the Amitrole draft Peer Review Document (Rinde to Yowell, 11/20/89) and adapted for Pronamide.

The following guidance is given in the Agency's Draft Policy Document (Thyroid Follicular Carcinogenesis: Mechanistic and Science Policy Considerations, SAB Review Draft, May 1988):

> "Studies over the last several decades in multiple laboratories and using a number of different treatment regimens (e.g., iodide deficiency) have demonstrated the significance of long-term thyroid-pituitary

hormonal imbalance in thyroid carcinogenesis. A consistent progression of events is noted: reduction in thyroid hormone concentrations, elevation in TSH levels, cellular hypertrophy and hyperplasia, nodular hyperplasia, and neoplasia. Hyperplasia and sometimes neoplasia of the pituitary may also be seen. A block in any of the early steps acts as a block for subsequent steps including tumor development, and cessation of treatment at an early stage in the progression results in regression toward normal thyroid structure and function. Based on these observations... the Agency concludes that:

i. thyroid follicular cell tumors may arise from long-term disturbances in thyroid-pituitary feedback under conditions of reduced circulating thyroid hormone and elevated TSH levels;

ii. the steps leading to these tumors are expected to show thresholds, such that the risks of tumor development are minimal when thyroid-pituitary homeostasis exists; and

iii. models that assume thresholds may be used to assess the risks of thyroid follicular cell tumors where there is evidence of thyroid-pituitary hormonal imbalance."

Two basic questions must be addressed before this policy is applied.

"The first is a qualitative issue which addresses whether it is reasonable to presume that the neoplasms are due to thyroid-pituitary imbalance. A corollary issue is the extent to which other carcinogenic mechanisms can be discounted. The second question concerns the procedures to be employed in estimating the risks of these agents."

"The answers to the first question allow one to assign chemicals producing thyroid tumors to one of three categories. The assignation is based upon knowledge as to whether the chemical disrupts thyroid-pituitary feedback, whether tumors other than thyroid follicular cell (and relevant pituitary) tumors are found, and whether mechanisms other than thyroid-pituitary imbalance may apply to the observed tumor response."

2. <u>Determination of whether neoplasms are due to thyroid pituitary imbalance</u>

The document goes on to describe the three factors which should be considered in making this determination (answering the first question, or "qualitative issue"). These are addressed as they apply to Pronamide as follows:

a. <u>Factor I. Consideration of whether the thyroid tumors associated with administration of Pronamide can be attributed to disruption of the thyroid-pituitary hormonal balance.</u>

The Policy states that six indicators should be considered in addressing this factor.

i. Goitrogenic activity in vivo:

In the 15-week thyroid special study in male rats, thyroid follicular cell hypertrophy/hyperplasia was observed but the incidences were the same for the mid dose (1000 ppm) and high dose (4000 ppm) groups. Thyroid follicular cell hypertrophy and/or hyperplasia was not observed in male rats in the chronic feeding/carcinogenicity study, but thyroid follicular cell hypertrophy was observed in female rats in the 12-month phase. Thyroid abs weight was not altered in either the special thyroid study or the chronic feeding/carcinogenicity study in rats, but an increase was observed in female dogs in a chronic 52-week study.

ii. Clinical Chemistry Changes (e.g., reduced thyroid hormone and increased TSH serum concentrations):

In the 15-week thyroid special study in male rats, serum T3 and rT3 were not reduced. Serum T4 was reduced in a dose-related manner. Except for a moderate (72%) increase observed after 4 weeks of treatment with the mid dose (1000 ppm), serum TSH was not significantly changed. This argues against the thyroid-pituitary imbalance as a mechanism for thyroid tumor formation, since a sustained increase in serum TSH is the "common factor" for thyroid neoplasia induced in experimental animals by a variety of chemical, physical, and/or dietary agents. There was no thyroid-related clinical chemistry information in the chronic feeding/carcinogenicity study in rats.

iii. Specific evidence of reduced hormone synthesis (e.g., inhibited iodine uptake) or increase thyroid hormone clearance (e.g., enhanced biliary excretion):

There was no information on thyroid hormone synthesis. There was no information on thyroid hormone clearance in the chronic feeding/carcinogenicity study in rats. In the 15-week thyroid study, thyroid hormone clearance was not investigated at the mid dose of 1000 ppm, the high dose level from the chronic carcinogenicity study at which thyroid adenomas were observed. However, a clear increase in thyroid hormone biliary clearance was observed at the high dose (4000 ppm).

iv. Evidence for progression (e.g., hypertrophy/hyperplasia, nodular hyperplasia - neoplasia:

Evidence of progression is limited. In the 15-week thyroid special study in male rats the incidences of thyroid follicular hypertrophy/hyperplasia were the same after either 4 or 15 weeks of treatment. In the chronic feeding/carcinogenicity study, there no evidence of progression in male rats but females showed thyroid follicular cell hypertrophy in the 12-month phase and

thyroid follicular cell adenomas in the 24-month phase. There was no evidence of progression to malignancy.

v. Reversibility of lesions after exposure is terminated:

There was no information on reversibility of lesions in the chronic feeding/carcinogenicity study in rats. In the 15-week thyroid special study in male rats, the incidence of thyroid hypertrophy/hyperplasia observed (10/10; 100%) after 4 weeks of treatment with the high dose (4000 ppm) was significantly reduced but not completely reversed 11 weeks after exposure termination (5/9; 56%). Alterations and subsequent reversal of TSH and other hormones would be expected with a thyroid tumorigen that operates via disruption of the thyroid-pituitary axis. However, such changes were not seen in the 15-week thyroid special study. There was no information on reversibility of lesions after treatment with the mid dose (1000 ppm).

vi. SAR to other thyroid tumorigens:

Pronamide is not structurally related to any of the known animal or human tumorigen.

b. Factor II. Consideration of the extent to which genotoxicity may account for the observed tumor effects.

Mutagenicity tests (gene mutation, structural chromosomal aberration, other genotoxic effects) on Pronamide were all negative.

c. Factor III. Evaluation of neoplasms in addition to thyroid follicular tumors, including pituitary tumors.

Pronamide caused liver carcinomas in male B6C3F1 mice. It also caused benign testicular interstitial cell tumors in male rats (see below).

d. Conclusions

As indicated above, based on the overall judgment of the Agency's six indicators, there is limited evidence that the thyroid tumors in the rat associated with administration of Pronamide may be due to a disruption of the thyroid-pituitary hormonal balance. No information on thyroid hormone synthesis is available and there was no investigation of lesion reversibility, T4 hepatic metabolism, and T4 biliary clearance with the high dose level from the chronic carcinogenicity study (1000 ppm; mid-dose level in this study), at which thyroid adenomas were observed.

3. Factors to be Considered in Determining Method to be Used in Estimating the Risks of Pronamide

Again, this guidance is taken from the Amitrole Peer Review Document and revised for Pronamide. The Committee considered these points when determining which method is to be used for estimating the carcinogenic risk for Pronamide.

Guidance given in the EPA DRAFT policy on Thyroid Neoplasia for proceeding with the quantitation of risk is as follows:

a. "Threshold considerations should be applied in dose-response assessments for those chemical substances where (1) only thyroid tumors (and relevant pituitary tumors) have been produced; (2) the tumors can be attributed to a disruption in thyroid-pituitary hormonal homeostasis; and (3) potential mechanisms other than thyroid-pituitary imbalance (e.g., genotoxicity) can be disregarded.

b. Special attention should be given to chemicals (1) that have induced thyroid tumors (and relevant pituitary rumors) that may be due to thyroid-pituitary imbalance, and (2) where there is also evidence of either a genotoxic potential or the induction of neoplasms at sites other than the thyroid (or pituitary). Generally, those cases will be approached using various principles laid out in the EPA Guidelines for Carcinogen Risk Assessment. A strong rationale must be articulated for handling these agents otherwise.

c. For those chemicals producing thyroid tumors that do not seem to be acting via thyroid-pituitary hormonal inhibition, dose-response assessments will be performed in accordance with the EPA Guidelines for Carcinogen Risk Assessment."

4. Threshold Model for Testicular Neoplasm

The Agency has no policy which implements a threshold model for testicular neoplasm. Endocrine function of the testis may or may not be regulated through a feedback mechanism similar to that controlling thyroid function, e.g. through an involvement of the anterior pituitary. Since this pilot study was conducted to test for such a mechanism of action, the 6 indicators used in assessing a threshold model for thyroid neoplasm will also be applied below to assess a theoretical threshold model for testicular neoplasm.

a. Testicular pathology in vivo:

In the 13-week pilot study in male rats, 4000 ppm Pronamide produced an increase in the number of testicular interstitial cells. However, this observation was stated to be equivocal because of "the small degree of change being evaluated and the possibility of producing this appearance through fortuitous tangential sectioning of seminiferous tubules." Testicular

pathology was not investigated at 1000 ppm, the high dose in the chronic feeding/carcinogenicity study in rats, at which benign testicular interstitial cell tumors were observed. There was no evidence of testicular hypertrophy and/or hyperplasia in the chronic feeding/carcinogenicity study. No alterations of testes weight were observed in either the 13-week pilot study in male rats or the chronic feeding/carcinogenicity study in rats. Absolute weight of the testes were slightly (<20%) increased in a subchronic study in rats.

b. Clinical chemistry changes (e.g., reduced testicular hormone and increased LH and FSH serum concentrations):

In the 13-week pilot study in male rats, serum testosterone was unaltered. Serum LH and FSH were increased both after 4 and 13 weeks of treatment with 4000 ppm Pronamide. Clinical chemistry changes were not investigated at 1000 ppm, the high dose in the chronic feeding/carcinogenicity study in rats, at which benign testicular interstitial cell tumors were observed. Pituitary and/or testicular-related clinical chemistry changes were not investigated in the chronic feeding/carcinogenicity study in rats.

c. Specific evidence of reduced testicular hormone synthesis or increase hormone clearance (e.g., enhanced biliary excretion):

There was no information on testicular hormone synthesis or clearance.

d. Evidence for progression (e.g., hypertrophy/hyperplasia, nodular hyperplasia - neoplasia:

Evidence of progression is very limited. In the 13-week pilot study in male rats, treatment with 4000 ppm for 13 weeks was associated with a controversial increase in the number of testicular interstitial cells. In the chronic feeding/carcinogenicity study in rats, testicular interstitial cell hypertrophy and/or hyperplasia was not observed prior to occurrence of adenomas. There was no evidence of progression to malignancy.

e. Reversibility of lesions after exposure is terminated:

There was no information on reversibility of lesions.

f. SAR to other testicular tumorigens:

Pronamide is not structurally related to any of the known animal or human carcinogens.

5. Conclusions

If a testis-pituitary hormonal control mechanism exists for the testis, then the evidence that Pronamide-induced testicular tumors in the rat may be related to a disruption in the testis-pituitary balance is very limited, based on the overall judgment of the 6 indicators. The PRC is advised that another 13-week testicular hormonal study is ongoing and will be available for review in the spring of 1993.

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